**Assessment of the Nutritional, Antioxidant and Antimicrobial Properties of *Chromolaena odorata* Leaves**

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**ABSTRACT**

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| **Background and Aims:** *Chromolaena odorata* is an invasive weed that grows all over Nigeria. Despite being regarded as an unwanted weed coupled with the rise in antibiotic resistance, there is a need to explore the nutritional and antimicrobial properties of the leaves. This study therefore evaluated the nutritional composition, antimicrobial, and antioxidant properties of *Chromolaena odorata* leaf extract  **Methodology:** *Chromolaena leaves* were collected, and the nutritional composition, phytochemical constituents, and antioxidant properties of *Chromolaena odorata* leaf extract were determined using standard procedures. The agar well diffusion method was used to evaluate the antimicrobial properties of the methanol and ethyl acetate extract against the pathogens. At the same time, the active compounds present in the leaf were identified using High-performance liquid chromatography (HPLC).  **Results:** The proximate compositions of the leaf are moisture content (9.06 ± 0.0376), ash content (1.45 ± 0.046), crude fat (6.88 ± 0.243), crude protein (6.88 ± 0.243), crude fiber (5.15 ± 0.074), and carbohydrate content (70.65 ± 0.148). The microelements were present in this order: K>Mg>P>Ca>Zn>Cl>Mn. Na and Cd had values of (0.01 ± 0.000), while Pb was not detected. Saponins, flavonoids, phenolics, steroids, and alkaloids except tannins were present. The total phenolic and flavonoid contents are 14.93 ± 0.11 mg GAE/g and 5.34 ± 0.04 mg QE/g, respectively. The 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide (NO) radicals, and thiobarbituric acid-reactive substance (TBARS) had IC50 values of 309.62 *µ*g/mL, 366.74 *µ*g/mL, and 572.17 *µ*g/mL, respectively, while the IC50 value for the ferric reducing antioxidant power (FRAP) was 271.25 *µ*g/mL. The leaf extracts of *Chromolaena odorata* showed varying degrees of inhibition against the tested pathogens. *Salmonella typhimurium* 14028 was the most susceptible bacterium, while *Escherichia. coli* 25922 showed the least activity (4.5 mm). The HPLC revealed the presence of active compounds, such as quercetin, chalcone, kaempferol, flavone, flavonol, naringenin, and chromomoric acid.  **Conclusion:** This study demonstrated the nutritional and dietary potential of *Chromolaena odorata* leaves. The extracts may be considered an effective antimicrobial agent against clinical pathogens. |

***Keywords:*** *Proximate, mineral composition, antioxidants, phytochemicals, antimicrobial*

1. INTRODUCTION

The application of medicinal plants in disease treatment has been practiced since time immemorial. These plants were known to be potent, but their active components remained unidentified until the advent of science. Some of these plants produce secondary metabolites and are used to treat gastrointestinal disorders and other conditions, such as diabetes, cancer, and microbial infections. Due to limited health coverage and poverty, these plants are common in sub-Saharan Africa, especially in rural areas. An example is *Chromolaena odorata* (L.) R.M. King & H. Robinson, belonging to the family Asteraceae, are recognized globally as a notorious, unwanted, and highly competitive weed. In Nigeria, it is known as Akintola taku, ewe Awolowo, or Independence leaf, while the Igbos refer to it as obu inenawa (Tiamiyu and Okunlade, 2020). The properties contributing to the ability of *C. odorata* include its high rate of nutrient assimilation, rapid reproduction, inhibition of other plant species, and survival in various soil and climate conditions (Olawale et al. 2022).

Some of the nutrients essential for healthy human growth and function are supplied by plants (Thangadarai et al., 2001). Fresh, edible plant leaves and stems are rich in protein and serve as an energy source for humans and animals (Tiamiyu and Okunlade, 2020). The high nutritional value of *C. odorata* leaves largely explains their consumption as a vegetable in southern Nigeria (Omokhua et al., 2016). The low fiber and extractable phenolic contents and high crude protein make *C. odorata* a potential feed for livestock (Sukanya et al., 2011). It was suggested that *C. odorata* might be used as a supplement to animal feed due to its caloric content, flavor, and nutrients that improve palatability (Mensah et al., 2008; Aro et al., 2009). Conversely, minerals are essential for proper nutrition, metabolic processes, acid-base equilibrium, osmolarity, bodily homeostasis, enhanced work capacity, and resistance to illness (Usunomena & Efosa, 2016). Aside from its antihypertensive, antispasmodic, antitrypanosomal, antiprotozoal, and antibacterial properties, the leaves are used in southern Nigeria to stop bleeding, dress wounds, and treat skin infections (Harini et al., 2014). Additionally, studies have shown its efficacy in treating colitis, diarrhea, malaria fever, toothaches, diabetes, skin conditions, and skin disorders (Odugbemi, 2006; Akinmoladun & Akinloye, 2007). Certain species of *C. odorata* native to Asia and Western Africa can help alleviate stomach aches (Omokhua et al., 2006) Paul et al. (2018) reported that the phenolic components in the extract of *C. odorata* leaves prevent stomach ulcers and internal bleeding from diathesis.

According to Kanase and Shaikh (2018), phytochemicals are plant substances with therapeutic, preventive, or defensive qualities. These phytochemical constituents are physiologically active in our body and responsible for the medicinal properties of *C. odorata* (Akinmoladun & Akinloye, 2007). Natural antioxidants support endogenous antioxidants in combating oxidative stress, which is crucial for both animal and human health. They prevent or limit the oxidation of substrates and protect cells from the damaging effects of reactive oxygen species (ROS), including hydroxyl radicals, singlet oxygen, and superoxide (Tiamiyu and Okunlade, 2020). Oxidative stress, caused by an imbalance between ROS and antioxidants, results in cellular damage (Gulcin, 2010).

Despite its reputation as a notorious weed, it is necessary to utilize this plant for beneficial purposes for both humans and animals. Therefore, this study aimed to determine the nutritional composition, phytochemical constituents, antioxidant, and antimicrobial properties of *C. odorata* leaves.

2. Material and methods

**2.1 Collection of Plant Material**

*Chromolaena odorata* leaves used in this study were collected inside the main campus of Ekiti State University, Ado Ekiti, Ekiti State, Nigeria. The leaves were cleaned, air-dried, and subsequently finely pulverized using an electric blender. The powdered samples were stored in a clean bag until ready for use. The plant was authenticated at the herbarium unit in the Department of Plant Science and Biotechnology.

**2.2 Proximate Analysis and Elemental Composition**

The proximate and mineral compositions of the leaves were determined by the methods described by AOAC (2000). Moisture content was determined using the air oven method after drying at 103-105 ºC. The ash content was determined by incinerating it in a muffle furnace until the sample turned to ash. The crude protein and fat were determined using the micro Kjeldah method and Soxhlet apparatus, respectively. The crude fibre was determined by taking the lipid-free sample obtained after ether extraction. Subsequently, the sample was serially heated with dilute acid and alkali to hydrolyze the digestible portion. The residual sample dried and weighed is the fiber content which is less the weight of the ash. The total carbohydrate was calculated as 100 - (Sum of the percentages of moisture, ash, fat, protein, and crude fiber). An atomic absorption spectrophotometer (AAS) was used in analyzing the mineral composition of the leaves (Na, Mg, K, Ca, P, Cl, Zn, Mn, Pb, and Cd) as stated by AOAC (2000)

**2.3 Preparation of the Extract**

Two hundred (200) grams of the sample was extracted in 2000 mL of distilled water, placed in a fitted conical flask, and shaken for 48 hours using a shaker at medium speed. The mixture was filtered using sterile Whatman paper No. 1. The filtrate, measuring up to 600 mL, was evaporated to dryness, and the resulting crude extract was subjected to subsequent analysis.

**2.4 Phytochemical Analysis**

2.4.1 Determination of Qualitative Phytochemicals

The qualitative assessment of tannins, saponins, flavonoids, terpenoids, and steroids present in the leaves was carried out using the protocols elaborated by Gul et al. (2017).

2.4.2 Determination of Quantitative Phytochemicals

The quantitative assessment of total phenolic and flavonoid contents was done using the method of Seidu & Otutu (2016).

2.4.3 Determination of Antioxidant Properties

The DPPH radical scavenging ability was assessed as described by Patil et al. (2009) and Pandey and Barve (2011), with minor modifications. The nitric oxide scavenging ability was assessed as stated by Borra et al. (2014), with gallic acid used as the positive control. The inhibition of these radicals was calculated in percentage terms. The ability to reduce ferric ions was evaluated following the methods stated by Benzie and Strain (1996) and Oyaizu (1986). Furthermore, the influence of the extract on the inhibition of thiobarbituric acid reactive substances (TBARS) production was assessed by the modified method of Niehaus and Samuelsson (1968). The percentage of TBARS inhibited was calculated.

**2.5 Antimicrobial Susceptibility Testing of *Chromolaena odorata***

2.5.1 Preparation of *Chromolaena odorata* Leaf Extract

*Chromolaena odorata* leaves were washed, air-dried, and then blended. Two hundred and fifty grams of dried plant material were extracted using 1200 mL of ethyl acetate and methanol. The mixture was subjected to irregular shaking before being left to stand for 48 hours. After 48 hours, the sample was concentrated under a vacuum at 40°C using a rotary vacuum evaporator and filtered using Whatman No. 1 filter paper. The resulting viscous semi-solid fluid extracts were kept for further studies.

2.5.2 Microorganisms

This research utilized four Gram-negative bacterial type strains: *Escherichia coli* (*E. coli* 25922)*, Klebsiella pneumoniae* (KPN 700303), *Salmonella typhimurium* (14028),and *Pseudomonas aeruginosa* (27853). These isolates were acquired from the Molecular Laboratory, Department of Pharmaceutical Microbiology, University of Ibadan, Oyo State.

2.5.3 Susceptibility Testing of Test Organisms

The isolates' broth cultures, adjusted to the 0.5 McFarland standard, were spread on sterile Mueller Hinton agar (Oxoid) plates. Wells were bored into the agar plates using a 6 mm cork borer, and various concentrations of plant extracts were introduced into the wells, along with the appropriate labels. Gentamicin, the positive control, was placed in one of the wells. Dimethyl sulfoxide, the negative control, ensured it did not affect the organisms. The plates were incubated for 24 hours at 37°C, and inhibitory zones were measured in centimetre using a ruler and this was converted to millimeters (Dauda et al., 2022). Values were recorded as average of triplicate readings.

2.5.4 Determination of Minimum Inhibitory Concentration (MIC)

The MIC was conducted using 96-well plates and the broth microdilution method. Extract samples were dissolved in double-strength Tryptone Soya Broth (MERK) to make a 100 mg/mL solution, which was then serially diluted to obtain concentration ranges of 50 mg/mL, 25 mg/mL, 12.5 mg/mL, and 6 mg/mL. Ciprofloxacin (10 µg/mL) was used as the reference for the antibacterial test. To determine growth or turbidity in the test plates, 10 µL of p-iodonitrotetrazolium violet (0.2 mg/mL) was added to each well for 30 minutes and further incubated at 37°C. Wells changing from yellow to pinkish-red indicated bacterial growth.

2.5.4 Determination of Maximum Bactericidal Concentration (MBC)

Ten microliters of the bacterial type strains were added to each microplate well and incubated for 24 hours at 37°C. The lowest concentrations showing no signs of growth or turbidity were streaked on nutrient agar. The MBC was the lowest concentration, showing no observed growth.

**2.6 Characterization of Bioactive Compounds using HPLC Analysis**

The qualitative-quantitative analysis of phenolic compounds in *C. odorata* leaves was determined using the method reported by Afolabi et al. (2019). Agilent 1100 series HPLC equipped with a diode array detector (DAD) (Agilent Technologies, Waldbronn, Germany) was analyzed using a 250 x 4.6mm, Knauer Eurosphere RP-18 analytical column with 5‐μm diameter particle size. A non-linear gradient elution was performed using 1% formic acid (solvent A) and methanol (solvent B). The analysis was carried out at 220°C and a flow rate of 1mL/min and 50 μL injection volume. The detection of the polyphenols according to their peaks was carried out between 220 and 550 nm wavelength and identified using retention time and UV spectra in comparison with standard phenolic compounds.

**2.7 Statistical Analysis**

The results were calculated and presented as mean ± standard deviation using Excel.

3. Results and discussion

**3.1 Proximate Analysis and Mineral Composition**

The proximate analysis showed the nutritional profile of the leaves. Carbohydrates had the highest composition value, while ash content was the lowest (Table 1). The evaluation of the elemental composition of *Chromolaena odorata* leaves is shown in Table 2. The study revealed the leaf's mineral profile, which was rich in potassium (110.39 ± 0.04), followed by magnesium (41.38 ± 0.17). Other essential minerals include calcium (10.27 ± 0.06), phosphorus (25.59 ± 0.02), and sodium (0.01 ± 0.00). While lead (Pb), a micronutrient, was not detected in the sample, other micronutrients were present in minute quantities.

**Table 1. Proximate composition of the Leaves of *Chromolaena odorata***

|  |  |  |
| --- | --- | --- |
| **S/N** | **Parameters** | **Values** |
| 1 | Moisture content (%) | 9.06 ± 0.07 |
| 2 | Ash content (%) | 1.45 ± 0.08 |
| 3 | Crude fat (%) | 6.84 ± 0.62 |
| 4 | Crude Protein (%) | 6.88 ± 0.42 |
| 5 | Crude fibre (%) | 5.15 ± 0.13 |
| 6 | Carbohydrate (%) | 70.65 ± 0.26 |

*\*Values are mean ± standard deviation of triplicate readings*

**Table 2. Mineral composition of the leaves of *Chromolaena odorata***

|  |  |  |
| --- | --- | --- |
| **S/N** | **Parameters** | **Values** |
| 1 | Sodium | 0.01 ± 0.00 |
| 2 | Magnesium | 41.38 ± 0.17 |
| 3 | Potassium | 110.39± 0.04 |
| 4 | Calcium | 10.27 ± 0.06 |
| 5 | Phosphorus | 25.59 ± 0.02 |
| 6 | Chloride | 0.07 ± 0.01 |
| 7 | Zinc | 0.37 ± 0.01 |
| 8 | Manganese | 0.02 ± 0.00 |
| 9 | Lead | ND |
| 10 | Cadmium | 0.01 ± 0.00 |

\**Values are mean ± standard deviation of duplicate readings*

**3.2 Phytochemical Screening of *Chromolaena odorata* Leaves**

The qualitative phytochemical analysis of *C. odorata*leaves is shown in Table 3. Tests for saponin, flavonoids, phenolics, steroids, and alkaloids were positive, while tannins were absent. The aqueous of *C. odorata*leaves extract exhibited a high content of total phenolics, quantified at 14.93 ± 0.11 mg GAE/g, and total flavonoids, quantified at 5.34 ± 0.04 mg QE/g (Table 4).

**Table 3. Qualitative Phytochemical Screening of *Chromolaena odorata* Leaves**

|  |  |
| --- | --- |
| **Phytochemicals** | ***Chromolaena odorata*** |
| Saponin | + |
| Total flavonoids | ++ |
| Total phenolics | + |
| Steroids | + |
| Tannins | - |
| Alkaloids | + |

\**Legend: + (present); - (absent).*

**Table 4. Total Phenol and Total Flavonoid Content of *Chromolaena odorata* Leaves**

|  |  |
| --- | --- |
| **Phytochemicals** | **Values** |
| Total Phenolic Content (mg GAE/g) | 14.93 ± 0.11 |
| Total Flavonoid Content (mg QE/g) | 5.34 ± 0.04 |

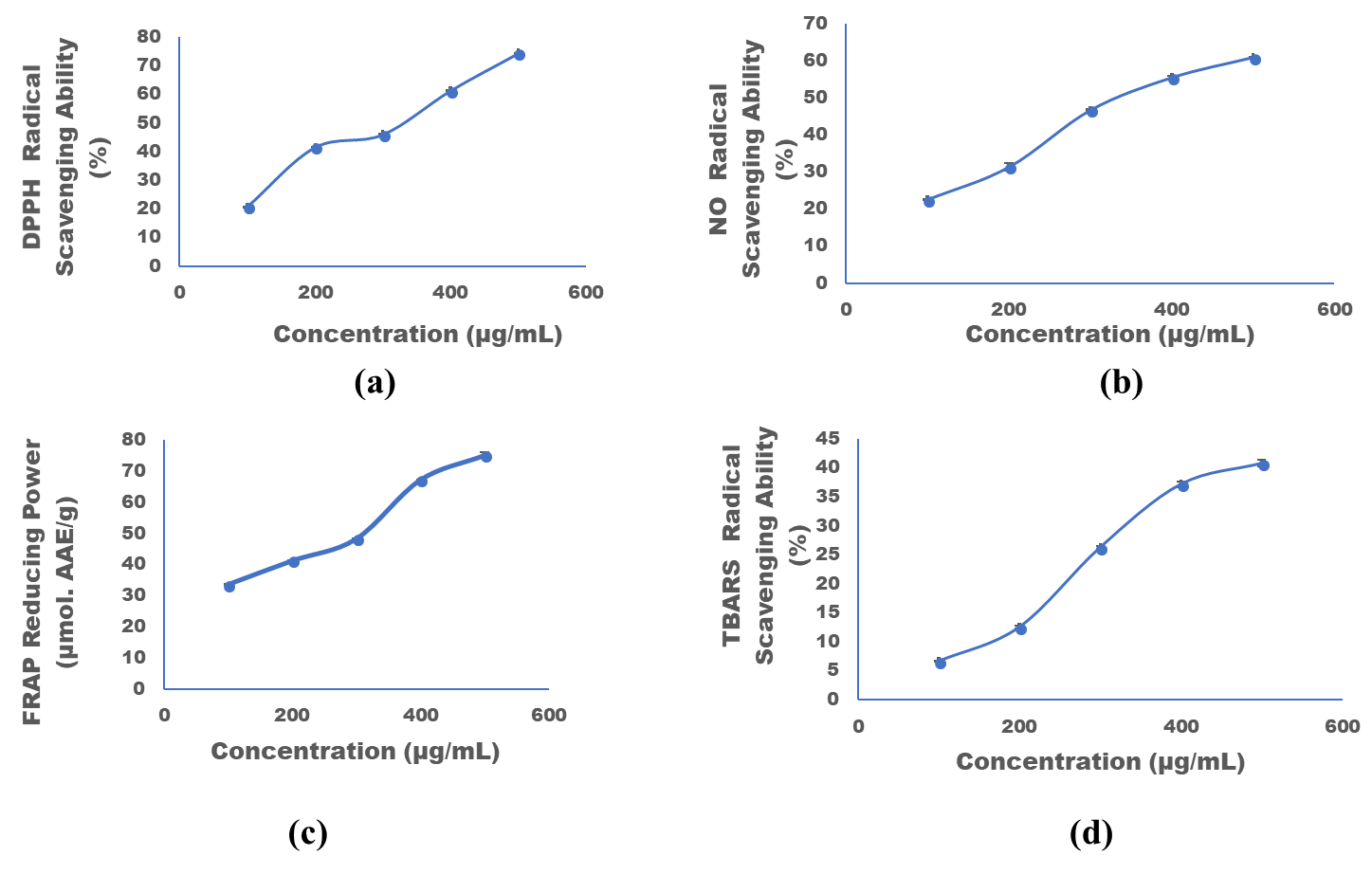
\**Values are mean ± standard deviation of duplicate readings.*

**3.3 Antioxidant Activities of *Chromolaena odorata* Leaves**

The DPPH, NO radical scavenging ability, and FRAP of the aqueous extract of leaves of *Chromolaena odorata* are presented in Fig. 1. *Chromolaena odorata*leaf extracts exhibited varying levels of antioxidant activity, with IC50 values observed for different assays. Specifically, the IC50 values for DPPH radical scavenging ability is 309.62 µg/mL, for nitric oxide (NO) is 366.74 µg/mL, FRAP (Ferric Reducing Antioxidant Power) is 271.25 µg/mL and for TBARS (Thiobarbituric Acid Reactive Substances) inhibition at 572.17 µg/mL.

**3.4 Antibacterial Activity of Methanolic and Ethyl Acetate Extract of *Chromolaena odorata***

The results of the evaluation of *Chromolaena odorata*'s methanolic and ethyl acetate extracts' antibacterial activity against four (4) clinical pathogens are shown in Table 5. Various concentrations of the plant extract demonstrated distinct antibacterial effects against the tested species of bacteria, depending on the concentration. Tables 5 and 6 show the minimum inhibitory concentration (MIC) of *Chromolaena odorata* methanolic and ethyl acetate extract needed to stop each test pathogenic strain from growing.

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**Fig. 1. Graph showing the (a) DPPH Radical Scavenging Ability (%) (b) NO Radical Scavenging Ability (%) (c) FRAP Reducing Power and (d) TBARS Radical Scavenging Ability (%) of Aqueous Extract *Chromolaena odorata* Leaves**

**Table 5. Antibacterial Activity of Ethyl Acetate Extract of *C. odorata* Leaves**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Isolates** | **Conc. (mg/ml)/Zone of Inhibition (mm)** | | | | | | |
|  | **100** | **50** | **25** | **12.5** | **Gent** | **MIC** | **MBC** |
| *Escherichia coli* 25922 | 10 | 8 | 6 | 5 | 15 | 50 | 100 |
| *Klebsiella pneumoniae* 700303 | 18 | 12 | 10 | 8 | 16 | 25 | 50 |
| *Salmonella typhimurium* 14028 | 23 | 20 | 18 | 16 | 12 | 25 | 25 |
| *Pseudomonas aeruginosa* 27853 | 20 | 18 | 15 | 12 | 16 | 25 | 50 |

**Table 6. Antibacterial Activity of Methanolic Extract of *C. odorata* Leaves**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Isolates** | **Conc. (mg/ml)/Zone of Inhibition (mm)** | | | | | | |
|  | **100** | **50** | **25** | **12.5** | **Gent** | **MIC** | **MBC** |
| *Escherichia coli* 25922 | 8 | 6 | 5 | 4.5 | 15 | 50 | 100 |
| *Klebsiella pneumoniae* 700303 | 15 | 12 | 10 | 8 | 16 | 50 | 100 |
| *Salmonella typhimurium* 14028 | 15 | 16 | 14 | 10 | 13 | 25 | 50 |
| *Pseudomonas aeruginosa* 27853 | 16 | 15 | 10 | 8 | 15 | 25 | 50 |

**3.5 HPLC-DAD Analysis of Phenolic Composition**

The HPLC chromatogram and phenolics of the crude extract of *Chromolaena odorata* (L.) R.M. King & H. Robinson are shown in Fig. 2 and Table 7, respectively. The HPLC characterization revealed the major phenolics as flavone, chalcone, flavonol, quercetin, and kaempferol. The most prominent compound, quercetin, was eluted at 11.05 min, followed by chalcone, while all eluted compounds were detected in the range of 1.27-17.616 min. These phenolic compounds have known potential as antioxidants, anti-inflammatory, antimicrobial, and antidiabetic, amongst other pharmacological properties.

**Fig. 2. HPLC Chromatogram of Crude Extract of *Chromolaena odorata***

**Table 7. HPLC Evaluation of Phenolic Compounds of Crude Extract of *Chromolaena odorata*** (L.) R.M. King & H. Robinson

|  |  |  |  |
| --- | --- | --- | --- |
| **S/N** | **Compound** | **Concentration (mg/g)** | **Retention time** |
| 1 | Flavone | 1243.780515 | 1.266 |
| 2 | Chalcone | 3019.330569 | 2.75 |
| 3 | Flavonol | 1002.645113 | 4.45 |
| 4 | Aurone | 498.8579 | 5.466 |
| 5 | Cadalene | 236.4760473 | 6.483 |
| 6 | Eupolin | 116.53284 | 7.95 |
| 7 | Quercetin | 59618.57529 | 11.05 |
| 8 | Kaempferol | 2531.617872 | 12.166 |
| 9 | Naringenin | 743.2481863 | 13.7 |
| 10 | Luteolin | 199.7286002 | 14.816 |
| 11 | Quercetagetin | 187.6539182 | 15.716 |
| 12 | Eupatilin | 117.6405829 | 16.25 |
| 13 | Rinderine | 135.5814609 | 17.233 |
| 14 | Chromomoric Acid | 662.1693826 | 17.616 |

This study was able to evaluate the nutritional composition, phytochemicals, antioxidant and antimicrobial properties, and bioactive compounds present in *C. odorata* leaves from the study area. The proximate analysis reveals the nutritional profile of any given food. This information could help to complement dietary supplements, animal feed composition, or drug development.

The nutritional composition of the *C. odorata* leaves showed a low moisture content and were a good source of fiber, protein, and carbohydrates. The moisture content of the leaves obtained here is similar to the moisture content (9.09 ± 0.72) reported by Etejere et al. (2017) and lower than the 8.50% reported by Archana et al. (2023) for *C. odorata* leaves. This implies that microbial activity will be retarded in the leaves because the lower the moisture content, the higher the shelf life of any given substance. In addition, the acceptable limits for most vegetable drugs are estimated at around 6 to 15%, which signifies that the value obtained in this study is within the limit (Kunle, 2000). A higher ash content value of 6.17% was reported by Archana et al. (2023), which was higher compared to that of this study. In comparison, crude lipid of 0.25% and 3.54 ± 0.14% was reported for *C. odorata* and *N*. *cordifolia* leaflets by Ngozi et al. (2009) and Oyeyemi et al. (2019), respectively. Fats are important for the protection and insulation of vital organs as well as the production of hormones (Dutta et al., 2005). Conversely, the protein value obtained in this study is higher than the 4.01% reported in the leaves of *Senna siamea* (Alli Smith, 2009). Protein is crucial in maintaining and repairing human tissues, synthesizing essential hormones, and providing energy when other sources are insufficient (Efosa et al., 2017). Protein also provides essential amino acids for proper nourishment (Oyeyemi et al., 2019). This indicates that the leaf can serve as a good source of protein.

Fiber contents of 26.78%, 26.57%, and 0.03 ± 0.01% were detected in *C. odorata* leaves, as reported by Archana et al. (2023), Ngozi et al. (2009), and Etejere et al. (2017), respectively. Dietary fibers reduce the risk of hyperglycemia, colon cancer, hyperlipidemia, cardiovascular disease, hypercholesterolemia, and type II diabetes (Eshak et al., 2010; Alahmari, 2024). This suggests that the leaves can be used as a dietary supplement. The carbohydrate content of the *C. odorata* leaves obtained in this study was higher than the 19.07 ± 0.5% reported by Etejere et al. (2017) and 50.82% observed by Ngozi et al. (2009). However, Efosa et al. (2017) reported that the leaves of *Irvingia gabonensis* O’Rorke Baill had a carbohydrate value of 75.15 ± 1.29%. Carbohydrates are essential nutrient that provides energy for different body functions and metabolism. This suggests that the leaf is a good source of carbohydrates. The proximate analysis implies that the *C. odorata* leaves analyzed in this study are good sources of fat, carbohydrates, energy, fiber, and protein needed to meet the minimum daily requirements.

The mineral profile showed that the leaves were rich in potassium while cadmium had the lowest value. However, Usunomena and Efosa (2016) reported the composition of Ca, Mg, K, and Na in *C. odorata* leaves as 487.40 ± 1.06, 116.70 ± 1.01, 96.91 ± 1.05, and 44.22 ± 1.02, respectively. Oyeyemi et al. (2019) also reported that *N*. *cordifolia* leaves were rich in potassium, while Omolola (2019) reported that magnesium was the highest in *T*. *diversifolia* leaves. The absence of Pb and a low level of Cd indicates the safety of the leaves. Minerals serve crucial roles in body systems. For instance, sodium is a vital component that regulates blood pressure and water distribution (Turan et al., 2003). Magnesium aids the proper functioning of the immune system, protein synthesis, energy metabolism, and neuromuscular conduction, among others (Al Alawi et al., 2021). Potassium is a cofactor in enzymatic processes and plays a role in water balance, muscular contraction, nerve impulse conduction, osmotic pressure regulation, and acid-base balance (Roche, 2016). This implies that the minerals Mg, Ca, K, and P required to achieve the minimal daily needs are in good amounts in the leaves.

Phytochemicals such as saponins, flavonoids, phenolics, steroids, and alkaloids were positive, while tannins were absent in the qualitative test. The total flavonoid and phenolic content were high, as shown in the quantitative analysis. The presence of these bioactive compounds in the leaves justifies the antimicrobial activity and antioxidant properties of the leaves. Ejiofor and Nna (2022) reported alkaloids, saponins, steroids, flavonoids, tannins, and triterpenoids in *C. odorata* leaves. Conversely, Etejere et al. (2017) reported the phenolic and flavonoid content of *C. odorata* leaves as 1.20 ± 0.18 mg GAE/g and 8.00 ± 0.97 mg QE/g, respectively. Ogunniran et al. (2023) reported 13.25 ± 0.03 mg GAE/g and 3.99 ± 0.01 mg QE/g as total phenolics and flavonoid contents for *Senna siamea* leaves. Plants' medicinal properties stem from their phytochemical constituents, which show distinct physiological effects on humans (Daniel, 1999). In addition to their analgesic and wound-healing qualities, saponins have been shown to possess anti-fungal, anti-tumor, and antiviral capabilities (Arawande et al., 2013). The presence of saponins in this study supports the cholesterol-lowering capabilities of *Chromolaena odorata* (Nwankpa et al., 2012).

Steroids have anti-inflammatory qualities that also regulate the metabolism of proteins and carbohydrates (Nielson & Cox, 2005). Flavonoids' in vitro antibacterial potency has been associated with their ability to bind with bacteria's soluble and extracellular proteins (Chauhan et al., 2013). Alkaloids are reported to be effective as antimalarial agents and analgesics (Nna et al., 2018). Phenolics are a dominant class of phytochemicals believed to account for most antioxidant action in plants (Thabrew et al., 1998). Medicinal plants are, by nature, potential antioxidants, and evidence of these properties has been obtained from various in vitro, in vivo, clinical, and in silico studies. The fact that several biological processes and environmental factors are reservoirs of oxidants makes the body develop different antioxidative mechanisms to balance the level of oxidation. These mechanisms involve the upregulation of antioxidant enzymes or molecules, free radical scavenging, and preventing and breaking chain reactions of peroxidation, which ultimately convert oxidants into weaker stable molecules and repair damaged biomolecules (Kiran et al., 2023). The primary function of the body’s endogenous antioxidant defense can become overwhelmed during pathological processes, necessitating the use of exogenous agents. The value of medicinal plants as exogenous antioxidants comes from their ability to directly or indirectly influence the body’s defense mechanism because of the presence of phytochemicals. This process prevents the development of oxidative stress and the associated diseases. The antioxidant potential of *Chromolaena odorata* is revealed in this study via its radical scavenging ability. The ability of the aqueous extract of the leaves of *C. odorata* to scavenge DPPH and NO radicals, reduce ferric ions, and prevent the generation of TBARS conforms with the study of Eze and Jayeoye (2021), where the phenolic extract of the leaves of *C. odorata* scavenged radicals. The lowest IC50 value observed from the ferric reducing power of *C. odorata* stands this plant out as a good reducing agent.

The outcomes demonstrated the efficacy of methanolic and ethyl acetate extracts against some bacteria. This observation aligns with the findings of Ohunayo et al. (2021), who reported that the methanol extracts of medicinal plants were active (low zone of inhibition) against *E. coli*. The ethanol extract of *C. odorata* leaves demonstrated an inhibitory activity of 6.5 to 16 mm and MIC of 0.13 to 8 mg/mL against Gram-positive and Gram-negative bacteria (Irobi, 1997). This investigation confirmed zones of inhibition against gram-negative bacteria. The zones of bacterial inhibition for the ethyl acetate and methanolic leaf extracts of *C. odorata* were determined to be 5-23 mm and 4.5-18 mm, respectively, indicating that the ethyl acetate leaf extract exhibited superior antibacterial activity compared to the methanolic extract.

The research of Naido et al. (2011) showed that methanol extracts from the leaves inhibited all gram-positive bacteria considered in the study and one gram-negative bacterium, *E. coli*, which is similar to our research. The leaf extracts of *C. odorata* inhibited the growth of *S. aureus* (17 mm), *E. coli* (15.3 mm), and *P. vulgaris* (12.3 mm) at 100% concentration (Godwin and Andrew, 2015). Also, Tiamiyu et al. (2023) reported that the leaf extract of *C. odorata* inhibited the growth of *P. aeruginosa* with zones of inhibition of 12.50 ± 1.26 mm (100%), 9.50 ± 0.96 mm (50%), and 6.00 ± 0.82 mm (25%). Additionally, Atindehou (2013) showed that *C. odorata* leaf extracts had antibacterial efficacy against *Salmonella enterica, Vibrio cholerae, Shigella sonnei*, and *Klebsiella oxytoca*, with MIC values ranging from 0.156 to 1.25 mg/mL. Few bacterial isolates in this investigation were resistant to the tested antibiotics in vitro, while the majority were either sensitive or intermediate. Since antibiotic resistance develops gradually over time, genetic alterations are likely to contribute to this phenomenon (Isichei, 2005). However, this process is being accelerated by the abuse and overuse of antibiotics. Antibiotics are frequently administered without a doctor's supervision and are overused and abused in both humans and animals in many regions. Administering antibiotics to animals and fish as growth promoters and treatment of viral diseases like the flu and cold are examples of antibiotic abuse (Headrick, 2021).

Similarly, the presence of bioactive compounds identified from the leaves of *C. odorata* correlates with the antioxidant properties observed in this study (Crespo and Duran, 2024). Quercetin, chalcone, and kaempferol were the top flavonoids quantified in the plant, and so far, these compounds stand out as well-known free radical scavengers (Chen et al., 2013; Okolo et al., 2021). Quercetin is highly recognized as the most potent flavonoid in nature, with the capacity to scavenge reactive oxygen species (Hadidi et al., 2022). Most importantly, they are good reducing agents and are known to regulate the level of a chief antioxidant molecule, which is glutathione. They catalyze the reduction of GSSG (oxidized glutathione) to GSH (reduced glutathione) (Qi et al., 2022). Being the compound with the highest concentration in the leaves of *C. odorata*, its ability to reduce oxidants conforms with the ferric-reducing capacity of the plant. Quercetin was similarly found in the methanolic root extract (Devi et al., 2022) of *C. odorata*. However, Olawale et al. (2022) opined that no similar compounds were identified in the methanol hydro-distilled extracts of the root.

4. Conclusion

The results show that *C. odorata* leaves are rich in protein, carbohydrates, fat, crude fiber, magnesium, potassium, calcium, and phosphorus, indicating significant nutritional potential. These leaves could be used as a dietary supplement. Since the leaves inhibited the growth of Gram-negative clinical pathogens, this suggests that the extracts may be used as an antimicrobial agent against clinical pathogens. This could potentially aid in combating the growing concern of antimicrobial resistance. Several species of plant are known to have pyrrolizidine alkaloid, a secondary metabolite used as a natural defense against predators. This compound is supposedly poisonous after consumption. Hence, further studies on the alkaloid fraction of *C odorata* leaves can be assessed for this compound.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

References

King, R. M., & Robinson, H. (1970). *Chromolaena odorata* (Linnaeus). In: Phytologia. Vol. 21. New York: Bronx Park; 544–545.

Tiamiyu, A. M., & Okunlade, O. A. (2020). Benefits and detriments of Siam weed (*Chromolaena odorata*): A review. Biochemistry and Biotechnology Research, 8, 21-28.

Olawale, F., Olofinsan, K., & Iwaloye, O. (2022). Biological activities of *Chromolaena odorata*: A mechanistic review. South African Journal of Botany, 144, 44-57. <https://doi.org/10.1016/j.sajb.2021.09.001>

Thangadarai, D., Viswanathan, M. B., & Ramesh, N. (2001). The chemical composition and nutritional evaluation of *Canavalia virosaa*: a wild perennial bean from Eastern Ghats of Penninsular India. European Food Research and Technology, 213, 456-459.

Omokhua, A. G, McGaw, L. J., Finnie, J. F., & Van Staden, J. (2016). *Chromolaena odorata* (L.) R.M. King & H. Rob. (Asteraceae) in sub-Saharan Africa: A synthesis and review of its medicinal potential. Journal of Ethnopharmacology, 183, 112–122. <https://doi.org/10.1016/j.jep.2015.04.057>

Sukanya, S.L., Sudisha, J., Prakash., H. S., & Fathima, S. K. (2011). Isolation and characterization of antimicrobial compound from *Chromolaena odorata* S. L. Journal of Phytology, 3, 6-32.

Mensah, J. K., Okoli, R. I., Ohaju-Obodo, J. O., & Eifediyi, K. (2008). Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. African Journal of Biotechnology, 7, 2304-2309.

Aro, S. O., Osho, I. B., Aletor, V. A., & Tewe, V. A. (2009) *Chromolaena odorata* in livestock nutrition. Journal of Medical Research, 3, 1253-1257.

Usunomena, U., & Efosa, G. E. (2016). Phytochemical analysis, mineral composition and in vitro antioxidant activities of *Chromolaena odorata* Leaves. ARC Journal of Pharmaceutical Sciences, 2, 16-20. <https://dx.doi.org/10.20431/2455-1538.0202003>

Harini, K., Showmya, J., & Geetha, N. (2014) Phytochemical constituents of different extracts from the leaves of *Chromolaena odorata* (L.) King and Robinson. International Journal of Pharmaceutical Sciences and Business Management, 2, 13-20.

Odugbemi, T. (2006). Outlines and pictures of medicinal plants from Nigeria. University of Lagos Press, Lagos, Nigeria. p. 1-283

Akinmoladun, A. C., & Akinloye, O. (2007). Effect of *Chromolaena odorata* on hypercholesterolemia related metabolic imbalances. Proc. Akure- Humbold Kellog/3rd SAAT Annual Conference, FUTA, Nigeria. p. 287-290.

Paul, T. S., Das, B. B., Ingale, S. P., Killedar, N., & Apte, K. G. (2018) Oral intake of polyphenols of *Chromolaena odorata*: A perspective in peptic ulcer, thrombocytopenia, and heparin-induced bleeding diathesis in rodent model. Pharmacognosy Research, 10, 426–431. <http://dx.doi.org/10.4103/pr.pr_107_18>

Kanase, V. A., & Shaikh, S. A. (2018). A Pharmacognostic and pharmacological review on *Chromolaena odorata* (L.) RM King & H. Rob. (Siam weed). Asian Journal of Pharmaceutical and Clinical Research 11, 34-38. <https://doi.org/10.22159/ajpcr.2018.v11i10.26863>

Gulcin, I. (2010). Antioxidant properties of resveratrol: A structure-activity insight. Innovative Food Science and Emerging Techniques, 11, 210-218. <https://doi.org/10.1016/j.ifset.2009.07.002>

AOAC (2000). Official Methods of Analysis, Association of Official Analytical Chemists, Washington DC, USA.

Gul, R., Jan, S.U., Faridullah, S., Sherani, S., & Jahan, N. (2017). Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from *Ephedra intermedia* indigenous to Balochistan. Scientific World Journal. 5873648 <https://doi.org/10.1155/2017/5873648>

Seidu, K. T., & Otutu, O. L. (2016). Phytochemical composition and radical scavenging activities of watermelon (*Citrullus lanatus*). Croatian Journal of Food Science and Technology, 8(2), 83-89 <https://doi.org/10.17508/CJFST.2016.8.2.07>

Patil, A. P., Patil, V. V., & Patil., V. R. (2009). In vitro free radicals scavenging activity of *Madhuca indica* Gmel. Pharmacology Online, 2, 1344-1352.

Pandey, N., & Barve, D. (2011). Antioxidant activity of ethanolic extract of *Annona Squamosa* Linn bark. International Journal of Research in Pharmacology and Biomedical Sciences, 2, 1692-1697.

Boora, F., Chirisa, E., & Mukanganyama, S. (2014). Evaluation of nitrite radical scavenging properties of selected Zimbabwean plant extracts and their phytoconstituents. Journal of Food Processing, 2, 1-7. <https://doi.org/10.1155/2014/918018>

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of ‘‘antioxidant power’’: The FRAP Assay. Analytical Biochemistry, 239:70–76. <https://doi.org/10.1006/abio.1996.0292>

Oyaizu, M. (1986). Studies on product of browning reaction: Antioxidative activities of browning reaction prepared from glucose amine. Japanese Journal of Nutrition, 44, 307-315. <http://dx.doi.org/10.5264/eiyogakuzashi.44.307>

Niehaus, W. G., & Samuelsson, D. (1968). Formation of Malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. European Journal of Biochemistry, 6, 126-130. <https://doi.org/10.1111/j.1432-1033.1968.tb00428.x>

Dauda, O. S., Ohunayo, A. S., Afolabi, A. T., John-Mese, O. J., & Adeleke, O. E. (2022). Comparative antibacterial activity of honey and gentamicin against *Klebsiella* species. Journal of Advances in Biology and Biotechnology, 25(8), 28-34. <https://doi.org/10.9734/jabb/2022/v25i8591>

Etejere, E. O., Olayinka, B. U., & Aderemi, R. O. (2017). Phytochemical analysis of aqueous extract and proximate composition of *Chromolaena odorata* (L.) R.M. King and H. Robinson. Centrepoint Journal (Science Edition), 23, 173-182.

Archana, C. M., Kaarunya, E., & Jenifer, A. A. (2023). Organoleptic study, microscopic evaluation, and fluorescence analysis of *Chromolaena odorata* (L.) King and Robinson. International Journal of Creative Research Thoughts, 11(12), 198-207.

Kunle, O. F. (2000). Phytochemical and microbiological studies of the leaf of *Lippia* *mutiflora* Mold., Fam Verbenaceae. Unpublished Ph. D dissertation of the Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Ngozi, I. M., Jude, I. C., & Catherine, I. C. (2009). Chemical profile of *Chromolaena odorata* L. (King and Robinson) leaves. Pakistan Journal of Nutrition, 8, 521-524. <https://doi.org/10.3923/pjn.2009.521.524>

Oyeyemi, S. D., Adebiyi, A. O., & Ojo, V. I. (2019). Phytochemical and nutritional evaluation of *Nephrolepis cordifolia* (L) C. Presl., a tropical edible fern. East African Scholars Journal of Biotechnology and Genetics, 1(4), 91-98.

Dutta, D., Chaudhuri, U. R., & Chakraborty, R. (2005). Structure, health benefits, antioxidant property and processing and storage of carotenoids. African Journal of Biotechnology, 4(13), 1510 -1520.

Alli-Smith, Y. R. (2009). Determination of chemical composition of *Senna siamea* (*Cassia* leaves). Pakistan Journal of Nutrition, 8, 119-121. <https://doi.org/10.3923/pjn.2009.119.121>

Efosa, G. E., Oboso, E. E., & Usunomena, U. (2017). Proximate composition, mineral content and amino acid profile of *Irvingia gabonensis* O’Rorke baill leaf. International Journal of Scientific World, 5(1), 23-27. <http://dx.doi.org/10.14419/ijsw.v5i1.6969>

Eshak, E. S., Iso, H., Date, C., Kikuchi, S., Watanabe, Y., Wada, Y., et al. (2010). Dietary fiber intake is associated with reduced risk of mortality from cardiovascular disease among Japanese men and women. The Journal of Nutrition, 140, 1445–1453. <https://doi.org/10.3945/jn.110.122358>

Alahmari, L. A. (2024). Dietary fiber influence on overall health, with an emphasis on CVD, diabetes, obesity, colon cancer, and inflammation. Frontiers in Nutrition, 11, 1510564. <https://doi.org/10.3389/fnut.2024.1510564>

Omolola, T. (2019). Phytochemical, proximate and elemental composition of *Tithonia diversifolia* (Hemsley) A. Gray leaves. International Annals of Science, 8:54-61. <https://doi.org/10.21467/ias.8.1.54-61>

Turan, M., Kordis, S., Zeyin, H., Dursan, A., & Sezen, Y. (2003). Macro and micro minerals contents of some wild edible leaves consumed in eastern Anatolia. Acta Agriculturae Scandinavica, Section B - Soil and Plant Science, 53, 129-137.

Al Alawi, A. M., Al Badi, A., Al Huraizi, A., & Falhammar, H. (2021). Magnesium: the recent research and developments. Advances in Food and Nutrition Research, (96), 193–218. <https://doi.org/10.1016/bs.afnr.2021.01.001>

Roche, J. R. (2016). Feed Ingredients: Feed Supplements: Macrominerals. Reference Module in Food Science. <https://doi.org/10.1016/B978-0-08-100596-5.00759-9>

Ejiofor, N., & Nna, P. J. (2022). Analysis of the phytoconstituents of *Chromolaena odorata* leaves and its bioactivities against some clinical and plant pathogens. FNAS Journal of Scientific Innovations, 3, 1-9.

Ogunniran, A. O., Dauda, O. S., Rotimi, D., Jegede, F. C., Falodun, D. J., & Adekunle, P. O. (2023). Nutritional, phytochemical, and antimicrobial properties of *Senna siamea* leaves. Toxicology Reports 2024. 13: 101793. <https://doi.org/10.1016/j.toxrep.2024.101793>

Daniel, M. (1999). Impediments preventing India becoming a herbal giant. Current Science, 87, 275-276.

Arawande, J. O., Komolafe, E. A., & Imokuede, B. (2013). Nutritional composition of fireweed (*Crassocephalum crepidioides*). International Journal of Agricultural Technology, 9, 371-381.

Nwankpa, P., Eteng, M. U., Oze, G., Nwanjo, H. U., & Ezekwe, S. (2012). Effect of *Chromolaena odorata* on serum lipid profile and oxidative stress status in *Salmonellae typhi* infested wistar rats. Annals of Biological Research, 3, 4696-4700.

Nielson, D. L., & Cox, M. M. (2005). Lenninger’s Principle of Biochemistry (4th ed.) Palgrave MacMillian/ W.H. Freeman Indian ed. New Delhi.

Chauhan, R., Ruby, K. M., & Dwivedi, J. (2013). Secondary metabolites found in *Bergenia* species: A compendious review. International Journal of Pharmacy and Pharmaceutical Sciences, 5, 9-16.

Nna, P. J., Egbuje, O. J., & Don-Lawson, D. C. (2018). Determination of phytoconstituents and antimicrobial analysis of the ethyl acetate extract of *Carica papaya* seed. International Journal of Research and Innovation in Applied Science, 4, 1-7.

Thabrew, M. I., Hughes, R. D., & McFarlane, I. G. (1998). Antioxidant activity of *Osbeckia aspera*. Phytotherapy Research, 12, 288-290.

Kıran, T. R., Otlu, O., & Karabulut, A. B. (2023). Oxidative stress and antioxidants in health and disease. Journal of Laboratory Medicine, 47(1), 1-11.  <https://doi.org/10.1515/labmed-2022-0108>

Eze, F. N., & Jayeoye, T. J. (2021). *Chromolaena odorata* (Siam weed): A natural reservoir of bioactive compounds with potent anti-fibrillogenic, antioxidative, and cytocompatible properties. Biomedicine and Pharmacotherapy, 141, 111811. <https://doi.org/10.1016/j.biopha.2021.111811>

Ohunayo, A. S., Dauda, O. S., John-Mese, O. J., Oyinlade, P. O., and Afolabi, A. T. (2021). Antimicrobial assay of methanolic extracts of selected plants on multiple antibiotic resistant *Escherichia coli*. Quest Journals: Journal of Research in Pharmaceutical Science, 7(9), 7-12.

Irobi, O. N. (1997). Antibiotic properties of ethanol extract of *Chromolaena odorata* (Asteriaceae). International Journal of Pharmacognosy, 35(2), 111-115. <https://doi.org/10.1076/phbi.35.2.111.13287>

Naidoo, K. K., Coopoosamy, R. M., & Naidoo, G. (2011). Screening of *Chromolaena odorata* (L.) King and Robinson for antibacterial and antifungal properties. Journal of Medicinal Plants Research, 5, 4859-4862.

Godwin, O. O., & Andrew, A. N. (2015). Phytochemical screening, mineral contents and antibiogram of *Chromolaena* *odorata* (L) King & Robinson. Journal of Biology and Nature, 4(2), 97–103.

Tiamiyu, A. M., Okocha, R. C., Okunlade, O. A., Olatoye, I. O., and Adedeji, O. B. (2023). Phytochemical constituents, nutritional and antibacterial potentials of selected medicinal plants. Traditional Medicine Journal, 28(1), 1-9. <https://doi.org/10.22146/mot.78700>

Atindehou, M., Lagnika, L., Guérold, B., Strub, J. M., Zhao, M., Van Dorsselaer, A., et al. (2013). Isolation and identification of two antibacterial agents from *Chromolaena odorata* L. active against four diarrheal strains. Advances in Microbiology, 3(1), 115-121. <http://dx.doi.org/10.4236/aim.2013.31018>.

Isichei, A. O. (2005). The role of plant resources in Nigeria's economic recovery agenda. Nigerian Journal of Botany, 18, 1-22.

Headrick, J. D. (2021). The FDA, its guidances and the industries it is supposed to regulate. Drake Journal of Agricultural Law, 21, 263-1107.

Crespo, A. J. C., & Duran, N. M. (2024). Characterization of bioactive compounds, antioxidants and antimicrobial properties of *Allagoptera leucocalyx*. Food Chemistry Advances, 5, 100775. <https://doi.org/10.1016/j.focha.2024.100775>

Chen, H. J., Lin, C. M., Lee, C. Y., Shih, N. C., Peng, S. F, Tsuzuki, M., et al. (2013). Kaempferol suppresses cell metastasis via inhibition of the ERK-p38-JNK and AP-1 signaling pathways in U-2 OS human osteosarcoma cells. Oncology Reports, 30, 925–932. <https://doi.org/10.3892/or.2013.2490>

Okolo, E. N., Ugwu, D. I., Ezema, B. E., Ndefo, J. C., Eze, F. U., Ezema, C. G., et al. (2021). New chalcone derivatives as potential antimicrobial and antioxidant agent. Scientific Reports, 11(1), 1-3. <https://doi.org/10.1038/s41598-021-01292-5>

Hadidi, M., Orellana-Palacios, J. C., Aghababaei, F., Gonzalez-Serrano, D.J., Moreno, A., & Lorenzo, J. M. (2022). Plant by-product antioxidants: Control of protein-lipid oxidation in meat and meat products. LWT Food Science and Technology, 169(3), 114003 <https://doi.org/10.1016/j.lwt.2022.114003>

Qi, W., Xiong, D., & Long, M. (2022). Quercetin: Its antioxidant mechanism, antibacterial properties and potential application in prevention and control of toxipathy. Molecules, 27(19), 6545 <https://doi.org/10.3390/molecules27196545>

Devi, G. B., Ramya, K. S., Sri, D. S., Josthna, P., & Naidu, C. V. (2022). Phytochemical screening study in different parts of *Chromolaena odorata* by LC-MS method and related parameters. International Journal of Science and Research Archive, 7(2), 128–140.

Afolabi, O. B., Oloyede, O. I., Agunbiade, O. S., Obafemi, T. O., Aline, B., Obajuluwa, A., et al. (2019). HPLC-DAD profiling and inhibitory potentials of ethylacetate and aqueous extracts of *Talinum triangulare* on key enzymes linked to type-2 diabetes (α-amylase and α-glucosidase) and oxidative stress (monoamine oxidase). Egyptian Journal of Basic and Applied Sciences, 6(1), 99–110.

Abbreviations

AOAC - Association of Official Analytical Chemists

DPPH - 2,2-diphenyl-1-picrylhydrazyl

FRAP - Ferric Reducing Antioxidant Power

GAE - Gallic Acid Equivalent

HPLC - High-Performance Liquid Chromatography

IC50 - Half Maximal Inhibitory Concentration

MBC - Minimum Bactericidal Concentration

MIC - Minimum Inhibitory Concentration

NO - Nitric Oxide

QE - Quercetin Equivalent

ROS - Reactive Oxygen Species

TBARS - Thiobarbituric Acid Reactive Substances